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REFINING THE RISK OF FREEZING MORTALITY FOR ANTARCTIC TERRESTRIAL MICROARTHROPODS

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Summary

In studies of three common, freezing susceptible, Antarctic microarthropods, the springtail *Cryptopygus antarcticus* and the mites *Alaskozetes antarcticus* and *Halozetes belgicae*, we report (i) the consequences on cold tolerance of cooling in contact with water, and (ii) the risk of freezing when held at temperatures above the typical freezing point (measured using standard techniques) for up to 12 h. The springtail showed no change in SCP distribution when in contact with freezing water while, in contrast, the mites showed clear shifts towards decreased cold tolerance, in addition to death of c. 33% of individuals during the freezing of the water. The springtail showed a bimodal SCP distribution, with the population divided into "high"(typically -8 to -12°C) and "low" (typically below -20°C) groups. Some animals held at temperatures above these values froze, over a timescale between minutes and several hours. These results highlight the danger of equating standard cold tolerance measures with mortality risk under more realistic water and thermal regimes.

Key words: Antarctic, mite, springtail, exogenous nucleation, mortality risk

INTRODUCTION

Many ecophysiological studies have used supercooling point (SCP) as a measure of cold tolerance. However, two major problems exist with applying the results of this approach to interpretations of an organism's field ecophysiology. The first is methodological, in that SCP may not be a fixed physical characteristic of the organism, as (i) measured SCP may vary as a function of the cooling rate applied to the organism (5) (hence, to allow comparisons, many studies use a "standard" rate of cooling of 1°C min^{-1}), and (ii) freezing is a stochastic process - a supercooled liquid or organism is not prevented from freezing; rather its probability of freezing is much reduced (e.g. 2). The second relates to interpretation, in that many organisms show deleterious effects, even death, when exposed to temperatures well above their SCP's (1).

Mites and springtails are the dominant terrestrial microarthropods in the maritime Antarctic. Ecophysiological aspects of their cold tolerance ability have been studied extensively in three common and representative species (the oribatid mites *Alaskozetes antarcticus* and *Halozetes belgicae* and the isotomid springtail *Cryptopygus antarcticus*).

These studies have concluded that all three species are freezing intolerant, as is typical for the higher taxa to which they belong (3, 7, 8, 13). There is very little evidence of mortality above their SCP (but see 6), and they are categorized as "highly chill tolerant" (*sensu* Bale (1)), if not "freeze intolerant" in its strict interpretation. Field samples of both species, especially those collected in summer, typically include individuals with a wide range of SCP's. These distributions are classified as bimodal. In the case of *C. antarcticus*, the majority of individual SCP's are found either in the range -8 to -12°C (*i.e.* they freeze at relatively high sub-zero temperatures, and are termed "high group") or below -20°C (freeze at low sub-zero temperatures, termed "low group") (see also 7, 11).

In typical field microhabitats, they are unlikely to experience temperature conditions approaching the extremes of their cold tolerance capacity, through active selection of microsites and passive buffering of these from winter extremes by snow (9, 10, 12). All three species are common in moist habitats. While the springtail is covered in fine hairs, and can float on water surfaces, the mites are often found submerged in pools and on waterlogged rocks or vegetation. However, their responses to ecologically realistic cold exposures - including prolonged exposure to sub-zero temperatures above their SCP, and possible nucleation from contact with other animals or surrounding water - have received little attention.

Using these representative species, we studied the consequences on the "standard" cold tolerance SCP measurement of cooling in contact with water, associated with the risk of freezing through external nucleation. We also examined, for *C. antarcticus*, the risk of freezing when animals were held at temperatures above the typical SCP of high or low group samples for up to 12 h.

METHODS

The studies were carried out at the British Antarctic Survey's Rothera Research Station, Adelaide Island (67°34'S, 066°08'W), on the western side of the Antarctic Peninsula. Cultures of both species were maintained in controlled environment facilities at the research station. Additionally, fresh material of *C. antarcticus* could be collected from field sites within 500 m of the laboratory.

Use of a differential scanning calorimeter (DSC) (4) allowed groups of 10-25 animals to be exposed together to the following regimes:

1a) Freezing in contact with water

Groups of each species were placed in contact with a 20µl water droplet in a DSC pan (springtails floating on the surface, mites submerged). The water was "seeded" with nucleating agents by filtering through locally-obtained vegetation, resulting in a freezing point of -3 to -5°C. The pan was sealed and subjected to a standard cooling regime of 1°C min^{-1}, during which exotherms from all animals freezing after the water droplet froze could be identified. For the mites, the number of individuals freezing when the droplet froze was then obtained by subtraction. The pans were reopened after completion of the cooling programme, to confirm mortality. The SCP distributions obtained were compared with control distributions obtained immediately, in the absence of water, from a separate group of animals from the same culture or field sample.

1b) Survival of external ice formation

In order to confirm that mites freezing within the water freezing event were killed, even though their individual freezing exotherms could not be detected within the much larger water

exotherm, three groups of 20 *A. antarcticus*, and two groups of 20 *H. belgicae*, were placed in contact with water, as above. These were cooled until completion of the freezing event (c. −5°C). The DSC was then reset immediately, the pans reopened and survivors counted.

2) Freezing when held at constant sub-zero temperature
Using field-fresh samples of *Cryptopygus antarcticus*, control SCP distributions were generated as above. Further groups of animals from the same sample were then exposed immediately in sealed pans in the DSC to constant temperatures of -5, -7 or -20°C for 12h. These temperatures were selected as being just above the upper boundaries of the high and low SCP groups of *C. antarcticus*. Individuals freezing during this period were identified by the occurrence of an exotherm.

RESULTS

1a) Freezing in contact with water
The springtail showed no change in SCP distribution when in contact with freezing water (Mann-Whitney U-test, p = 0.43) (Figure 1). In contrast, both mites showed clear upward shifts in SCP distribution (*A. antarcticus*, p = 0.02 to 0.002; *H. belgicae*, p = 0.0007). Additionally, c. 33% of individuals of both mite species froze within the water freezing event, at a temperature well above the control SCP range.

1b) Survival of external ice formation
Numbers of animals surviving the water freezing event are given in Table 1. Under the conditions of this experiment, mortality during the freezing event (*i.e.* at very high sub-zero temperatures) was significant for these two species.

Table 1. Proportion of animals surviving freezing of water surrounding them at high sub-zero temperatures (-3 to -5°C) (n = 20 animals per trial).

Mite species	n trials	Proportion surviving freezing of surrounding water
Alaskozetes antarcticus	3	35%, 45%, 65%
Halozetes belgicae	2	75%, 65%

(2) *Freezing when held at constant sub-zero temperature*
Control SCP distributions of the springtail were typically bimodal. Significant mortality (freezing) was observed when individuals were held for 12 h a few degrees above these temperatures, at -7 or -20°C, but not at -5°C (Table 2). The majority of freezing events (48% at -7°C and 67% at -20°C) occurred within 30 min of being held at the trial temperature (Fig. 2). However, remaining individuals froze after more extended exposure, up to 11.5 h after commencement of the trial.

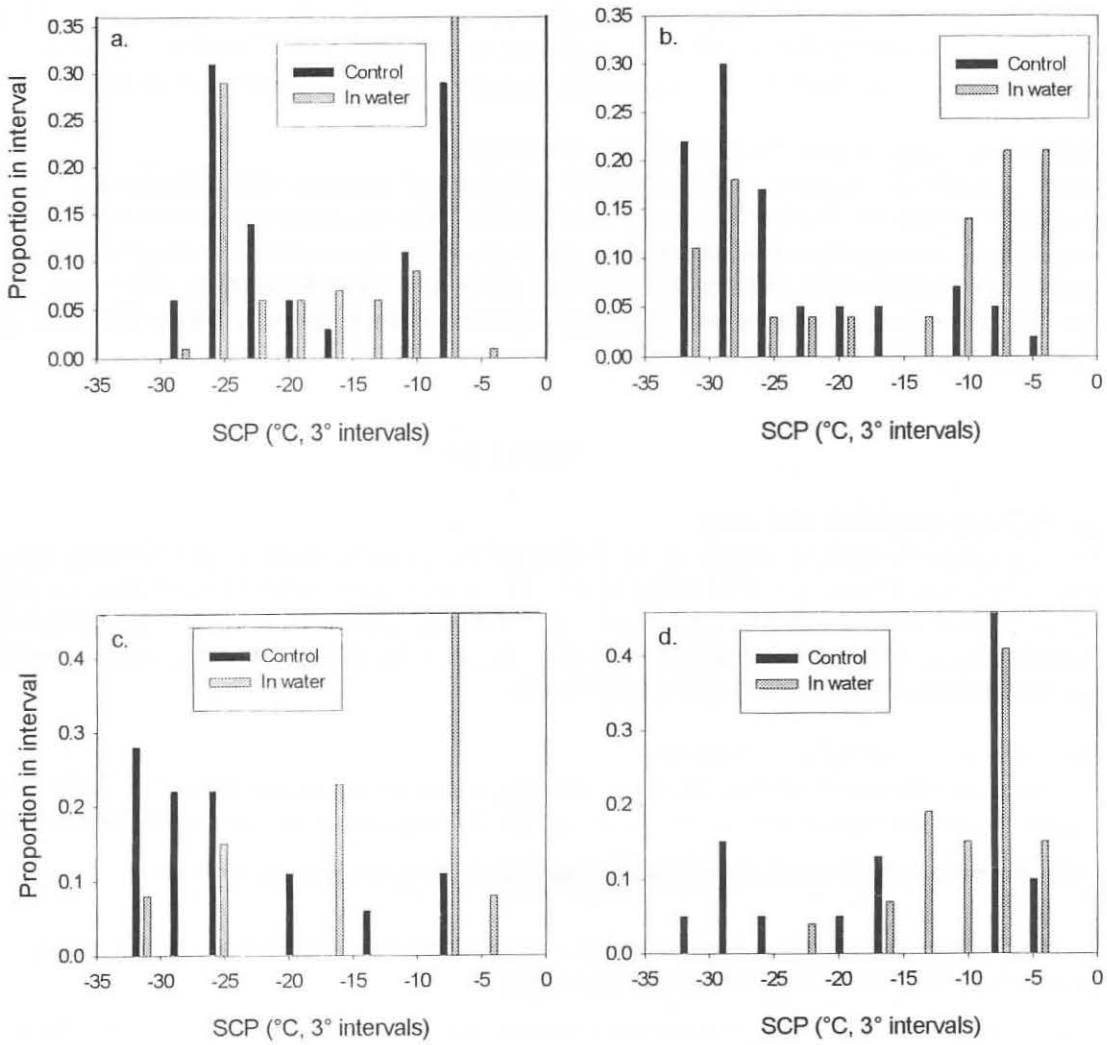

Figure 1. The influence of contact with water on population SCP distributions (a) *Cryptopygus antarcticus*; (b) *Halozetes belgicae;* (c) and (d) *Alaskozetes antarcticus* (two separate series of observations were made with *A. antarcticus*, as the control samples were obtained at different times and gave differing SCP distributions).

Table 2. The risk of freezing in *Cryptopygus antarcticus* when held at sub-zero temperatures above the standard SCP for 12 hours (n = 13-20 animals per trial)

Trial temperature (°C)	Percentage of control sample in high group	Percentage of experimental sample freezing when held at trial temperature
-5 (n = 2 trials)	52.6	0
	90	0
-7 (n = 3 trials)	31.6	25
	61.1	100
	62.5	63.6
-20 (n = 1 trial)	23.1	63.6

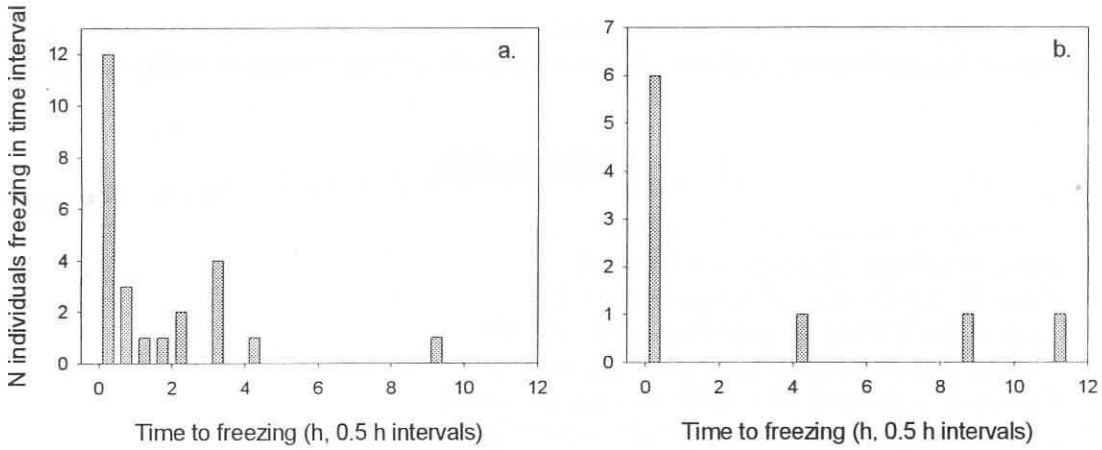

Figure 2. The time to freezing of individual *Cryptopygus antarcticus* when held at a constant temperature of (a) -7°C or (b) -20°C (see Methods; individuals freezing while the temperature was being reduced to the constant level are not included).

DISCUSSION

As observed for many springtails, *C. antarcticus* often rafts on water, contact being limited to the tips of body setae. The results of this study indicate this tactic is sufficient to ensure that the springtail does not face an increased risk of ice nucleation from contact with the water as it freezes. In contrast, however, *A. antarcticus* and *H. belgicae* show a much increased risk of exogenous nucleation in such situations (Table 1). Both these species are commonly found in moist or submerged situations, often in dense aggregations. A previous study of *A. antarcticus* has reported considerable survival over a period of 21 days of individuals encased in distilled water ice (6). Although that study has not been repeated, and no studies have been made of *H. belgicae*, it is commonly assumed that these oribatid mites are well adapted to avoid exogenous ice nucleation. The current observations indicate that the mites face a much

greater risk of death through freezing of surrounding water than has been recognized previously.

Likewise, *C. antarcticus* may face a greater risk of freezing under field conditions than previously recognized. Typically in summer, this springtail shows a bimodal SCP distribution (7, 11), with the majority of animals being in the higher group, while in winter the majority of animals are in the lower group. In the current study, some animals held at temperatures above these ranges froze at -7 or -20°C, over a timescale between minutes and several hours. Air temperatures below -5°C are experienced over short periods during the Antarctic summer, and are frequent during the winter. The demonstration that individuals may freeze after as little as a few minutes' exposure to the experimental temperatures indicates a significant risk of mortality under the temperature regimes experienced in the field, although the risk is likely to be reduced in winter through insulation by snow cover. These results also confirm that *C. antarcticus* should be described as highly chill tolerant rather than freeze intolerant in terms of Bale's (1) classification.

This study highlights the danger of equating cold-tolerance measures obtained using standard protocols with mortality risk under more ecologically realistic thermal and field hydration regimes. They further indicate that application of physiological studies of cold tolerance to the three species' ecology will result in refinement of current views of the role of cold tolerance in their life history strategies.

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